For general laboratory use.

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High Pure Viral RNA Kit

Version: 19

Content version: September 2017

For the isolation of viral RNA for RT-PCR

Cat. No. 11 858 882 001 1 kit
up to 100 purifications

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Binding Buffer	Contains 4.5 M guanidine-HCl, 50 mM Tris-HCl, 30% Triton X-100 (w/v), pH 6.6 (+25°C).	▪ 2 × 25 ml
2		Poly (A)	For binding of RNA	▪ 2 mg poly(A) carrier RNA (lyophilizate)
3a	black	Inhibitor Removal Buffer	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C) (final concentration after addition of ethanol)	▪ 33 ml, add 20 ml absolute ethanol
3	blue	Wash Buffer	20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) (final concentrations after addition of ethanol)	▪ 2 × 10 ml, add 40 ml absolute ethanol to each vial
4	colorless	Elution Buffer	Water, PCR grade	▪ 30 ml
5		High Pure Filter Tubes	For use of up to 700 µl sample volume.	▪ 2 bags with 50 polypropylene tubes with two layers of glass fiber fleece
6		Collection Tubes		▪ 8 bags with 50 polypropylene tubes (2 ml).

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

ⓘ *The buffers can show a slight yellow color. This will have no impact on the function of the buffer.*

1.2. Storage and Stability

Storage Conditions (Product)

⚠ ***The Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable until the expiration date printed on the label.***

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) will adversely impact nucleic acid purification due to formation of precipitates in the solutions.***

Storage Conditions (Working Solution)

Solution	Storage
Poly(A) carrier RNA solution	−15 to −25°C

1.3. Additional Equipment and Reagents Required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile

1.4. Application

The High Pure Viral RNA Kit is designed to purify intact viral RNA from serum or plasma samples. Viral RNA is used for RT-PCR analysis directly after elution in PCR grade water.

⚠ ***RNA preparations obtained are suitable for RT-PCR; they are not tested for other applications.***

1.5. Preparation Time

Total time	Approx. 20 min
Hands-on time	<10 min

2. How to Use this Product

2.1. Before you Begin

Sample Materials

200 – 600 µl research samples, such as serum, plasma, urine, or cell culture supernatant.

Control Reactions

⚠ ***It is the user's responsibility to implement an appropriate experiment control concept.***

General Considerations

Handling Requirements

⚠ ***Binding Buffer and Inhibitor Removal Buffer contain guanidinium hydrochloride which is an irritant.***
⚠ ***Do not allow Binding Buffer or Inhibitor Removal Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagent, dilute the spill with water before wiping it up.***
⚠ ***Do not use any modified ethanol.***
⚠ ***Do not pool reagents from different lots.***
⚠ ***Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening store all bottles in an upright position.***
⚠ ***Do not allow the Binding Buffer and Inhibitor Removal Buffer to mix with sodium hypchlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

Safety Information

Laboratory Procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

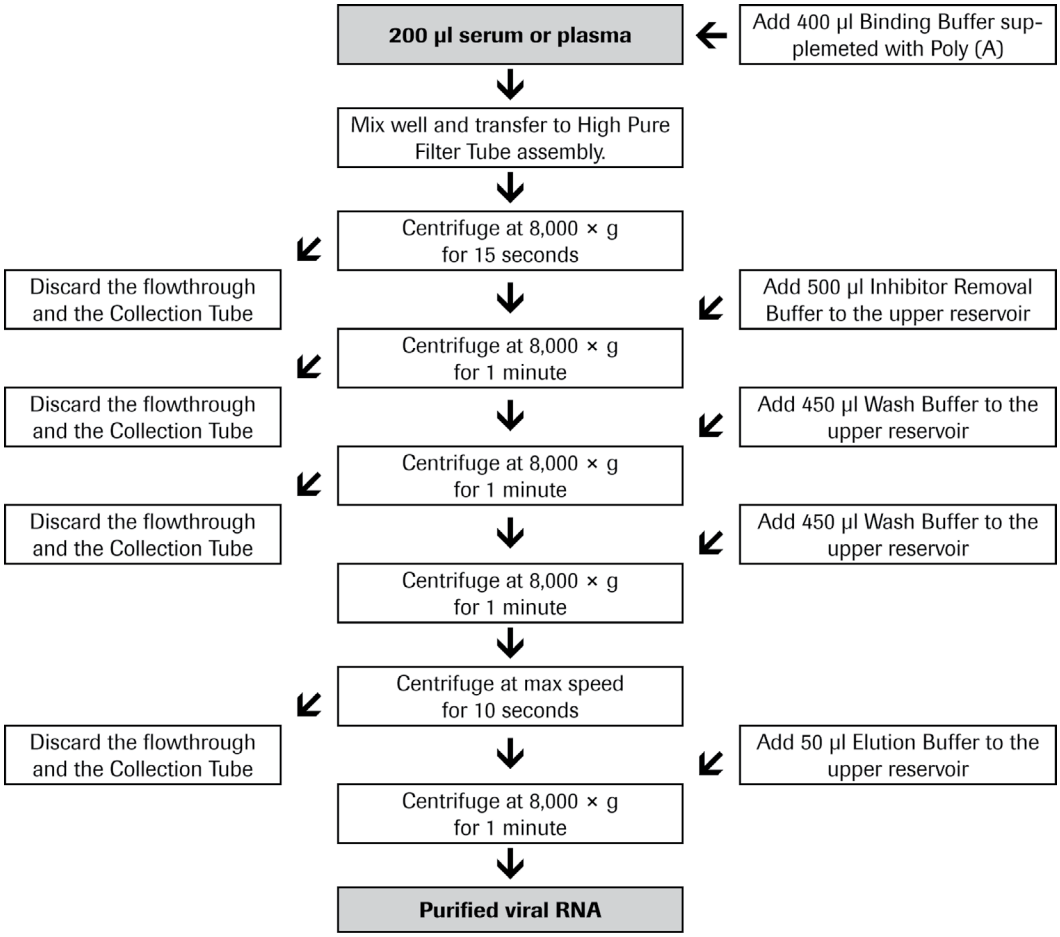
Working Solution

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content	Reconstitution/Preparation	Storage and Stability	For use in...
Poly (A) carrier (Vial 2)	Dissolve poly(A) carrier RNA (vial 2) in 0.4 ml Elution Buffer (vial 4). Prepare aliquots of 50 µl for running 8 × 12 purifications. Prepare aliquots of 100 µl for running 4 × 25 purifications.	▪ Store at −15 to −25°C. ▪ Stable for 12 months	For the preparation of working solution
	For 12 purifications, thaw one vial with 50 µl poly(A) carrier RNA and mix thoroughly with 5 ml Binding Buffer (vial 1). For 25 purifications, thaw one vial of 100 µl poly(A) carrier RNA and mix thoroughly with 10 ml Binding Buffer (vial 1)	⚠ ***Prepare always fresh before use! Do not store!***	Protocol Step 1
Inhibitor Removal Buffer (Vial 3a; black cap)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer. *Label and date bottle accordingly after adding ethanol.*	▪ Store at +15 to +25°C. ▪ Stable through the expiration date printed on kit label.	Protocol Step 5: To remove PCR inhibitors
Wash Buffer (Vial 3; blue cap)	Add 40 ml absolute ethanol to Wash Buffer. *Label and date bottle accordingly after adding ethanol.*	▪ Store at +15 to +25°C. ▪ Stable through expiration date printed on kit label.	Protocol Step 6 and 7: Removal of residual impurities

2.2. Protocols

Experimental overview



Isolation Protocol

Protocol for Preparing Viral RNA from 200 µl Serum or Plasma Sample.

⚠ ***If larger sample volumes (up to 600 µl) are to be used increase all components accordingly and load to the Filter Tubes multiple times. The number of total reactions of the kit decreases when larger samples volumes are processed.***

1. To a nuclease free 1.5 ml microcentrifuge tube:
   – Add 200 µl serum or plasma.
   – Add 400 µl Working solution [Carrier RNA supplemented Binding Buffer] and mix well.

   The RNA yield can be increased twofold by an optional incubation step, thus resulting in higher sensitivity. After adding the Binding Buffer to the sample, simply incubate the mixture at +15 to +25°C for 10 min. This incubation step can be omitted when time to result is critical.

2. To transfer the sample to a High Pure Filter Tube:
   – Insert one High Pure Filter Tube in one Collection Tube.
   – Pipette entire sample into the upper reservoir of the Filter Tube

3. – Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   – Centrifuge the tube assembly 15 sec at 8,000 × *g*.

4. After centrifugation:
   – Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
   – Insert the Filter Tube into a new Collection Tube.

5. After re-inserting the Filter Tube:
   – Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly and centrifuge 1 min at 8,000 × *g*.
   – Discard flowthrough and combine Filter Tube with a new Collection Tube.

6. After removal of inhibitors:
   – Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge 1 min at 8,000 × *g* and discard the flowthrough.

7. After the first wash and centrifugation:
   – Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
   – Insert the Filter Tube into a new Collection Tube.
   – Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge 1 min at 8,000 × *g*.
   – Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 sec at maximum speed (approximately 13,000 × g) to remove any residual Wash Buffer.

   The extra centrifugation time ensures removal of residual Wash Buffer.

8. Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.

9. To elute the viral RNA:
   – Add 50 µl Elution Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge the tube assembly for 1 min at 8,000 × *g*.

10. The microcentrifuge tube now contains the eluted viral RNA.
    Either use the eluted RNA directly in RT-PCR or store the eluted RNA at −80°C for later analysis.

    Use 3.5 – 6 µl of the eluate for the reverse transcription reaction.

3. Results

Serial dilutions of purified MS2 phage RNA were applied to the filter tubes, washed and eluted following the kit protocol. 3.5 µl of the 50 µl eluate were analyzed by two-step RT-PCR, using primers that resulted in a fragment of 961 bp. The numbers of RNA molecules per PCR reaction indicated in the figure below correspond to the assumed quantitative recovery.

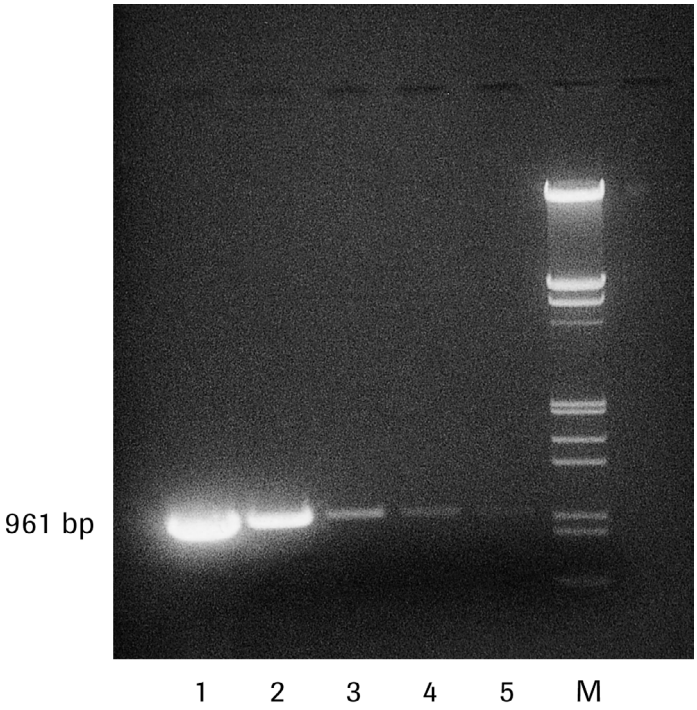


Fig. 1: RT-PCR analysis of MS2 RNA isolated with the High Pure Viral RNA Kit.

Lane 1: 3.5 × 10^7 molecules / PCR
Lane 2: 3.5 × 10^5 molecules / PCR
Lane 3: 3.5 × 10^3 molecules / PCR
Lane 4: 3.5 × 10^2 molecules / PCR
Lane 5: 35 molecules / PCR
Lane M: DNA Molecular Weight Marker III

Furthermore, the kit was used to prepare genomic RNA from viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in RT-PCR.
All these templates produced highly specific PCR products in good yield.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to condi-tions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
		After any lyophilized reagent is constitut-ed, aliquot it and store the aliquot at −15 to −25°C.
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer.	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always mark Wash Buffer vial and Inhibi-tory Removal Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Poor elution of nucleic acids with water	Water has the wrong pH.	If you use your own water or buffer to elute nucleic acids from Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbance ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light.	① Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 min at maximum speed. ② Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Low RNA yield	High levels of RNase activity.	Be careful to create an RNase-free working environment
		Process starting material immediately or store it at −80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at −80°C.

5. Additional Information on this Product

5.1. Test Principle

As a pre-requisite for the analysis of viral RNA by the reverse transcription polymerase chain reaction (RT-PCR) the isolation of the analyte from serum or plasma is required.
The High Pure Viral RNA Kit accomplishes virus lysis by incubation of the sample in a special Binding Buffer. Subsequently nucleic acids bind specifically to the surface of glass fibers in the presence of a chaotropic salt (guanidine-HCl). The binding reaction occurs within seconds due to the disruption of the organized structure of water molecules and the interaction of nucleic acids with the glass fibers surface. Thus, adsorption to the glass fiber fleece is favored. Since the binding process is specific for nucleic acids, the bound nucleic acids are purified from salts, proteins and other impurities by a washing step and are finally eluted in low salt Elution Buffer or PCR grade water. The purified viral RNA is free of intact virus, nucleases, and all cellular components that interfere with RT-PCR and can be applied directly for RT-PCR. 50 µl eluate is sufficient for 8-14 RT-PCR reactions.
Included in the kit is a special Inhibitor Removal Buffer resulting in improved sensitivity and reproducibility of RT-PCR assays performed with nucleic acid templates isolated with this kit. Especially, the use of the Inhibitor Removal Buffer allows even the application of heparinized sample material containing 100 U/ml heparin.

The High Pure Viral RNA Kit
- saves time, because the kit does not require extraction with organic solutions or nucleic acid precipitation and thus can prepare multiple RT-PCR templates in approximately 10 minutes
- accommodates a wide variety of samples, because the same kit can purify viral RNA from several bodily fluids
- minimizes RNA loss, because the kit removes contaminants without precipitation or solvent extraction
- increases lab safety, because the kit minimizes the handling of potentially hazardous samples and does not use hazardous organic solvents

① Serum or plasma are lysed by incubation with Binding Buffer.

② Nucleic acids are bound to the glass fibers pre-packed in the High Pure Filter Tube.

③ Bound nucleic acids are washed with a special Inhibitor Removal Buffer to get rid of RT-PCR inhibitory contaminants. It allows even the application of heparinized sample material with >100 U/ml heparin.

④ Washing of bound nucleic acids, purification from salts, proteins and other cellular impurities.

⑤ Purified nucleic acids are recovered using the Elution Buffer.

5.2. Quality Control

Series of MS 2 RNA dilution are prepared, applied to the filter tubes, washed and eluted following the kit protocol. 3.5 µl of the eluate is analyzed by RT-PCR. The products are detected on agarose gel. At least 2×10^5 RNA molecules / 200 µl sample are guaranteed.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Editorial changes.
Layout changes.

6.3. Trademarks

HIGH PURE is a trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.5. Regulatory Disclaimer

For general laboratory use.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

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